

IT IS CLAIMED

1. A human cell composition for use in producing one or more cytokines, comprising:
a human cell line characterized by expression of the coding sequence for an anti-apoptotic protein and a level of cytokine production that is at least two times (2X) the level of cytokine production exhibited by a corresponding parental cell line that does not express the coding sequence for the anti-apoptotic protein.

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10 2. The cell line composition according to claim 1, wherein said anti-apoptotic protein is CrmA.

15 3. A human cell composition for use in producing one or more cytokines, prepared by the process of:

(a) obtaining a parental human cell line capable of producing one or more cytokines;
15 (b) modifying the cells by introducing a first expression vector comprising the (i) coding sequence for CrmA operably linked to a first promoter, (ii) a first selectable marker-encoding nucleic acid sequence, and (iii) additional control elements necessary for expression in human cells, into the cells of said cell line; and
20 (c) culturing said modified cells in medium containing a first selection agent to select for CrmA-expressing cells.

25 4. A human cell composition prepared by the process of claim 3, further comprising the step of:

(d) treating said CrmA-expressing cells in a manner effective to result in enhanced cytokine production, wherein said transformed and treated cell line is characterized by a level of cytokine production that is at least two times (2X) the level of cytokine production by the corresponding non-transformed parental cell line.

30 5. A human cell composition prepared by the process of claim 3, further comprising the step of:

(d) further modifying said CrmA-expressing cell line by introducing a second expression vector comprising (i) the coding sequence for PKR operably linked to a second promoter; (ii) a second selectable marker-encoding nucleic acid sequence; and (iii) additional control elements necessary for expression in human cells into the cells of said cell line; and

35 (e) culturing said further modified cells in medium containing a selection agent specific for said second selectable marker to select for PKR overexpressing cells.

40 6. A human cell composition prepared by the process of claim 5, further comprising the step of:

(f) treating said PKR overexpressing cells in a manner effective to result in enhanced cytokine production, wherein said transformed and treated cell line is characterized by a level of

cytokine production that is at least two times (2X) the level of cytokine production by the corresponding non-transformed parental cell line.

7. A human cell composition prepared by the process of claim 4 or 6, wherein treating
5 means subjecting said transformed cells to one or both of priming and inducing.

8. The human cell composition according to claim 7, wherein priming means exposing
said transformed cells to phorbol myristate acetate (PMA) or interferon- β .

10 9. The human cell composition according to claim 7, wherein inducing means exposing
said transformed cells to a microbial inducing agent selected from the group consisting of Sendai
virus, encephalomyocarditis virus and Herpes simplex virus.

15 10. The human cell composition according to claim 9, wherein said microbial inducing
agent is Sendai virus.

20 11. The human cell composition according to claim 7, wherein inducing means exposing
said cells to at least one non-microbial inducing agent selected from the group consisting of
poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate,
cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

12. The human cell composition according to claim 11, wherein inducing means
exposing said cells to polyI:C, cycloheximide and Actinomycin D.

25 13. In an improved method for producing one or more cytokines in human cell culture,
the improvement directed to increasing cell viability and the amount of cytokine production, by
culturing a parental human cell under conditions of one or more of (i) modification effective to
result in anti-apoptotic protein expression; (ii) modification effective to result in cytokine
regulatory factor overexpression; (iii) priming; and (iv) inducing, wherein the amount of cytokine
30 production is at least two times (2X) the level of cytokine production by the corresponding non-
transformed parental cell line.

35 14. The method according to claim 13, wherein modification effective to result in anti-
apoptotic protein expression means introducing a first expression vector comprising the (i)
coding sequence for CrmA operably linked to a first promoter, (ii) a first selectable marker-
encoding nucleic acid sequence, and (iii) additional control elements necessary for expression of
CrmA in human cells into the cells of said cell line, and culturing the cells in medium containing
a first selection agent to select for CrmA-expressing cells.

40 15. The method according to claim 14, wherein modification effective to result in
cytokine regulatory factor overexpression means introducing a second expression vector
comprising (i) the coding sequence for PKR operably linked to a second promoter; (ii) a second

selectable marker-encoding nucleic acid sequence; and (iii) additional control elements necessary for expression in human cells into cells of said CrmA-expressing cell line, and culturing the cells in medium containing a selection agent specific for said second selectable marker to select for PKR-overexpressing cells.

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16. The method according to claim 14 or 15, further comprising priming the cells by exposing them to one or both of phorbol myristate acetate (PMA) and interferon- β .

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17. The method according to claim 15 or 16, further comprising inducing the cells by exposing them to a microbial inducing agent selected from the group consisting of Sendai virus, encephalomyocarditis virus and Herpes simplex virus.

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18. The method according to any one of claims 16 or 17, further comprising inducing the cells by exposing them to at least one non-microbial inducing agent selected from the group consisting of poly(I):poly(C) (poly IC), or poly r(I):poly r(C) (poly rIC), heparin, dextran sulfate, cycloheximide, Actinomycin D, sodium butyrate, a calcium ionophore and chondroitin sulfate.

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19. The method of according to claim any one of claims 17 or 18, wherein the one or more cytokine(s) are selected from the group consisting of interferon-alpha (IFN-alpha), interferon-beta (IFN-beta), interferon-gamma (IFN-gamma); granulocyte macrophage colony stimulating factor (GM-CSF); granulocyte colony stimulating factor (G-CSF); interleukin-2 (IL-2); interleukin-3 (IL-3); interleukin-7 (IL-7); interleukin-8 (IL-8); interleukin-10 (IL-10); and interleukin-12 (IL-12).

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